Rather, a more likely pathway—and one that should be tested in future studies—is one wherein ethanol metabolism by hepatocytes generates paracrine signals that drive 2-AG synthesis by stellate cells.

Paracrine stimulation of CB_1 receptor signaling in hepatocytes by 2-AG derived from neighboring stellate cells is a major finding of the Jeong et al. study. The importance of hepatocyte responses to 2-AG was confirmed first by using rimonabant to attenuate CB_1 signaling and thereby block steatosis in vivo, and then by assessing the effects of ethanol in mice selectively deficient in CB_1 receptors only in hepatocytes. These liver-specific CB_1 receptor knockout mice are particularly useful in that they avoid any confounding effects of CB_1 signaling in the central nervous system, where the receptor is far more abundantly expressed than in liver. Animals with hepatocyte-specific deletion of CB_1 receptors were resistant to the steatotic effects of ethanol feeding. Moreover, induction of the lipogenic mediators sterol regulatory element-binding protein 1c (SREBP-1c) and fatty acid synthase (FAS) was blunted and activity of carnitine palmitoyltransferase 1 (CPT1) was no longer inhibited in mice with either global or hepatocyte-specific deletion of CB_1 receptors. It would be informative to determine whether CYP2E1 expression is additionally affected by CB_1 loss, as this could attenuate liver injury by reducing oxidative stress.

The intriguing findings of Jeong et al. (2008) introduce a new paradigm in our understanding of fatty liver and its potential attenuation by available pharmacological agents. Paracrine signaling by a nonparenchymal cell to modulate hepatocyte responses may be relevant to a number of intermediary pathways apart from fat metabolism, including homeostasis of carbohydrates, proteins, vitamins, and metals (especially iron and copper). Effects of Kupffer cells and sinusoidal endothelial cells as other sources of paracrine stimuli should also be considered in order to dissect whether these effects derive exclusively from stellate cells, although this seems unlikely. It is possible, but still unproven, that the paracrine pathway described by Jeong et al. (2008) could contribute to fatty liver due to etiologies other than alcohol, especially NAFLD.

In summary, the uncovering of paracrine cannabinoid signaling as a determinant of hepatic steatosis unveils exciting new possibilities for both understanding and regulating fat accumulation in liver. Combined with data implicating cannabinoids in hepatic fibrogenesis, this pathway is assuming a central role in the regulation of hepatic metabolism, injury, and fibrosis.

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PPARγ: Ally and Foe in Bone Metabolism

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Bone homeostasis is a well-balanced process that is largely dependent on the contribution of both bone-forming osteoblasts and bone-resorbing osteoclasts. A new study (Wan et al., 2007) suggests a previously unsuspected role for the transcription factor PPARγ in promoting bone progenitors to the osteoclastic lineage.

The Italian anatomist Niccolò Massa undoubtedly appreciated the paramount importance of bones when he wrote as early as 1559 that "if any one is ignorant of the structure of the bones it follows necessarily that he will be ignorant of very many other things along with them." Several centuries later, bone is still a prime subject of modern biomedical research for many reasons, including that bone diseases, such as osteoporosis, osteopetrosis, arthritis, osteosarcoma, and others, are

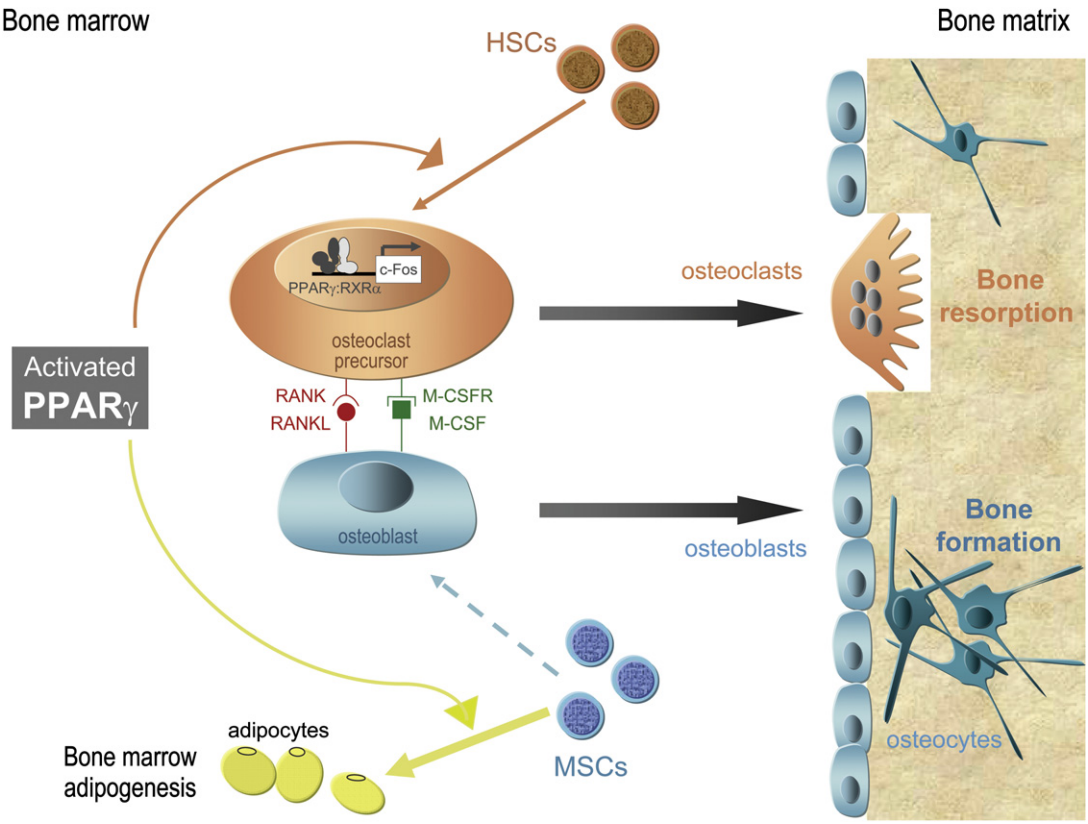

Figure 1. Effects of PPARγ on Bone Cell Differentiation

Deletion of PPARγ in mouse osteoclast precursors causes osteopetrosis due to impaired osteoclast differentiation from hematopoietic stem cells (HSCs). PPARγ ligand activation by rosiglitazone promotes osteoclast differentiation. In osteoclast precursor cells, activated PPARγ forms a heterodimer with retinoid X receptor α (RXRα) and enhances c-*fos* expression, which then stimulates osteoclastogenesis (Wan et al., 2007). Conversely, PPARγ downregulates osteogenesis by driving competition between adipogenic and osteoblastic differentiation of bone marrow progenitors (MSCs) in favor of adipogenesis (Akune et al., 2004; Cock et al., 2004). Osteoblasts are supporting cells for osteoclast differentiation that express the membrane-bound osteoclastogenic cytokine receptor activator of NF-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF). Osteoclast precursors express receptors for RANKL (RANK) and for M-CSF (M-CSFR, macrophage colony-stimulating factor receptor), through which osteoblasts signal to osteoclast precursors to impact on their fate.

serious and painful diseases that can impair the mobility of patients and thus their independence.

Osteoblasts and osteoclasts are essential in controlling the amount of bone tissue. Healthy bones undergo dynamic remodeling throughout life, being reshaped by the osteoblasts, which are responsible for bone formation, and the osteoclasts, which resorb bone by removing both its organic and inorganic components. A new role for the peroxisome proliferator-activated receptor γ (PPARγ) in the maintenance of bone homeostasis has emerged, as illustrated by the recent work of Evans and colleagues on osteoclastogenesis (Wan et al., 2007). Previously recognized key roles of this ligand-activated nuclear receptor were mainly in glucose and fat metabolism and in inflammation. The antidiabetic thiazolidinediones (TZDs) rosiglitazone and pioglitazone are agonists of PPARγ. These drugs are extremely well tolerated and have become popular in the treatment of type 2 diabetes. However, they have been much debated because a rare side effect of TZD treatment, fluid retention, can worsen congestive heart failure. Consequently, TZDs are not recommended for treatment of diabetic patients with moderate or severe heart failure.

Furthermore, in several clinical trials, including A Diabetes Outcome Progression Trial (ADOPT), which involved 4360 type 2 diabetes patients, an unexpected higher rate of fractures has been observed in groups that received rosiglitazone or pioglitazone treatment (Grey, 2008; Kahn et al., 2006). In animal studies, TZD treatments have demonstrated decreased bone formation and bone mass (Grey, 2008). These outcomes, which suggest that bone is vulnerable to antidiabetic therapies with PPARγ agonists, raised an important clinical issue regarding the potential role of PPARγ in bone loss.

In their recent study, Wan et al. (2007) report a previously unrecognized role of PPARγ in promoting osteoclast differentiation and bone resorption when activated by rosiglitazone (Figure 1). In this work, mice with a deletion of PPARγ in osteoclasts, but not in osteoblasts, developed a phenotype of increased bone mass and density and extramedullary hematopoiesis. This phenotype corresponds to the clinical syndrome called osteopetrosis, first described by the German radiologist Heinrich Albers-Schönberg in 1904, which is characterized by impaired bone resorption, resulting in skeletal fragility despite increased bone mass. Wan, Chong, and Evans (Wan et al., 2007) also used the converse approach, which consisted of a gain of PPARγ function by activating

the receptor with rosiglitazone. This treatment accelerated osteoclast differentiation and bone resorption in a PPARγ-dependent manner. In a series of elegant experiments, the molecular pathway by which PPARγ exerts its osteoclastogenic effect was revealed. PPARγ and its ligands promote osteoclast differentiation by controlling the c-*fos* gene, whose product was identified previously as a key regulator of the macrophage/osteoclast lineage and bone remodeling (Grigoriadis et al., 1994). The results of Wan et al. (2007) show that PPARγ deficiency selectively inhibits the c-*fos* arm of the membrane-bound receptor activator of NF-κB ligand (RANKL) signaling pathways.

An intriguing observation reported a few years ago was that osteoclasts cannot be derived from marrow macrophages unless they are cocultured with osteoblasts or their stromal cell precursors (Novack and Teitelbaum, 2008). Osteoblasts express RANKL and macrophage colony-stimulating factor (M-CSF), key osteoclastogenic cytokines (Figure 1). The crucial role of osteoblasts in osteoclastogenesis, via these cytokines, raises the question of whether PPARγ inhibits osteoblast differentiation directly or indirectly, which would thus confer on this nuclear receptor an overall control function determining the fate of the two major bone cell populations.

Osteoblasts and adipocytes are both generated from multipotent mesenchymal stem cells (MSCs) in bone marrow, and PPARγ is now recognized as a key adipogenic factor (Figure 1). Interestingly, age-related osteoporosis is concomitant with increased marrow adipose tissue. In a cell culture assay, PPARγ-deficient embryonic stem cells spontaneously differentiated into osteoblasts and failed to produce adipocytes, indicating that the receptor favors adipogenesis over osteoblastogenesis (Akune et al., 2004) (Figure 1). In mice, PPARγ insufficiency increases bone mass due to enhanced osteoblastogenesis from bone marrow progenitors, suggesting that the receptor functions as a suppressor of commitment to the osteoblastic lineage (Akune et al., 2004; Cock et al., 2004). Thus, PPARγ acts on bone metabolism by stimulating and inhibiting the osteoclastogenic and osteoblastogenic pathways, respectively (Figure 1). Importantly, Wan et al. (2007) genetically separated the effect of PPARγ on the hematopoietic lineage, which produces osteoclasts, from its effect on the mesenchymal lineage, which gives rise to osteoblasts. This was achieved by crossing Tie2Cre mice with homozygous PPARγ floxed mice, causing PPARγ ablation only in the hematopoietic lineage, which includes endothelial cells, bone marrow, thymus, spleen, and lymph nodes, without affecting the mesenchymal lineage. This enabled the investigators to analyze PPARγ function in only one of the two cell populations, the osteoclasts. However, this approach does not allow definitive dismissal of the possibility, although unlikely, that PPARγ deletion in other hematopoietic cells also affects the osteoclast progenitor cell population. This question will be answered when targeted deletion exclusively in osteoclasts becomes possible. Furthermore, Wan et al. used synthetic PPARγ ligands, and it remains to be investigated which, how, and when natural biological PPARγ ligands interfere with the large degree of plasticity of osteoclast as well as osteoblast progenitor cells.

Taken together, these recent findings on the roles of PPARγ should inspire new approaches to preventing metabolic deregulation in bones and treating bone diseases associated with increased bone loss. The potential for the pharmacological targeting of progenitor cells to increase bone regeneration is also reinforced by the finding that the differentiation of preosteoblasts to osteoblasts can be increased by the proteasome inhibitor bortezomib. This effect was observed in multiple myeloma patients suffering from bone lesions due to osteoclast-activating factors released by the tumor cells, which stimulate osteoclasts to break down bone (Giuliani et al., 2007). In mice, this antimyeloma proteasome inhibitor induces mesenchymal stem/progenitor cells, which can differentiate into bone, fat, or muscle, to preferentially undergo osteoblastic differentiation (Mukherjee et al., 2008).

In conclusion, the recent advances in osteoclast and osteoblast biology open opportunities for the exploration of combination therapies with agents that promote regenerative functions and bone maintenance by stimulating osteoblast differentiation and/or by inhibiting osteoclastic bone destruction. Furthermore, if such agents have antineoplastic activity, they may be beneficial for multiple myeloma patients with severe bone disease. New knowledge such as that gained through the study of Wan et al. (2007), when combined with PPARγ cell-type-selective modulators still to be developed, will win over PPARγ as an ally rather than a foe by promoting solely its beneficial effects on altered bone metabolism.

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